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Growth and Yield Responses of Rice to Acid Rain. I. Sensitivity to Composition and pH of Simulated Acidic Medium[1]

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Abstract : This study investigated the differences of physiological responses of rice (*Oryza sativa* L. cv. Tainung No.67) seedling to a variety of compositions and acidities of simulated acid rain (medium). Roots of Two to three weeks old seedings were soaked into simulated acidic media of different ratios(1:1, 5:1, and 1:5)of sulphuric acid (H_2SO_4) and nitric acid (HNO_3) for 1 h. To examine the pH effect, the level of acidic medium was adjusted to 5.0, 4.5, 4.0, 3.5, 3.0, or 2.5 using pure water treatment as control. Results showed that pH of the homogeneous solution of rice leaves was reduced from pH 6.69 to pH 6.45−6.42 when treated with simulated acidic media of pH 4.0. A 22- 24% of increase of electrical conductivity was found when the pH of acidic media lowered down to 3.5. The acidic buffering capacity of the homogeneous solution was built up 13−15% more as pH of acidic media further dropped down to 3.0. In the homogeneous solution of roots, its pH was reduced from 6.55 to 6.44-6.41 at pH 4.5 of simulated acidic media. When pH lowered to 4.0, the electrical conductivity went up 12-16%. When reached pH 3.5, the acidic buffering capacity was increased 31- 35%. As the results indicated, roots of rice seedings were more sensitive to external pH changes of simulated acidic medium than leaves, but the magnitude of response was inconsistent. At the same level of pH no statistically significant difference was found within acidic medium treatments of different compositions.

Key words : Rice seedling, Simulated acid rain, pH, Electrical conductivity, Acidic buffering capacity, Homogeneous solution.

INTRODUCTION

Natural rainwater has an pH of ca. 5.6 because of solution of atmospheric carbon dioxide and the formation of carbonic acid. By this fact, acid rain is defined as rain with a pH lower than 5.6. Due to air pollution problem there is evidence indicating an increase of the acidity of rain in many parts of the world over the past 40 years[6,18,19]. At present, there

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are no reports of direct injury of crops caused by acid rain. However, it has been reported that simulated acid rain at pH levels lower than in ambient rain induced physiological and morphological changes in various plant species. For instance, Evans *et al.*[10] showed that fewer pods and hence less seeds per plant were produced in soybean treated with simulated acid rain at pH lower than 4.0. At pH 3.1, dry mass of stems and leves were decreased[9]. Smith *et al.*[32] further pointed out that, though fewer flowers and pods were found at pH under 4.0, no physiological basis for soybean yield responses to acid rain of pH 3.0 was identified. In contrast, Irving and Miller[15] found no effect of simulated acid rain of pH 3.1 on soybean yield components. Norby and Luxmoore[22] showed no significant difference in leaf weight of soybean treated with simulated acid rain of pH 2.6. Norby *et al.*[23] did not find any effect of simulated acid rain of pH 3.4 on dry matter distribution of soybean. Thus, experimental results show an inconsistent response to acidic solutions up-to-date.

On physiological responses, numerous traits have been recorded from simulated acid rain treatments, including disintegration of leaf cuticles[8], increased leaching of nutrients from leaves[11,12,37], chlorosis or necrosis of leaves[8,17], and loss of diurnal behavior of stomata [39]. Few information, however, is available on the physiological response of leaf and root of a crop to a short-term ambient acidity.

SO_2 and NO_2 are two common atmospheric pollutants and are known to be toxic to vegetation. They may be incorporated in clouds and be deposited later in rainfall as dilute sulphuric and nitric acids. In fog water collected at Los Angeles area of USA, Waldman *et al.*[36] found it to have higher acidity(pH 2.2-4.0) and higher concentrations of sulfate, nitrate, and ammonium. Sun and Wu[33] evidenced that the average pH value of rainfall measured in many regions of Taiwan was about 4.2 and SO_4^{-2} and NO_3^- were the two major components. Chen and Chen[6] further reported that the average raito of SO_4^{-2}/NO_3^- in Taiwan area was about 4.

The effect of acid rain on crops may be induced by direct incorporation of acidic solution to the aboveground plant parts and/or adsorption of harmful ions by roots. After entering the plants, the reactions can be physiologically or morphologically. As little is known about the physiological response of rice to acid rain, this study investigated the differences of cytoplasmic pH, electrical conductivity, and acidic buffering capacity of rice leaf and root by direct soaking roots in siumulated acid rain (medium) of various compositions and acidities. Their physiological implications were discussed.

MATERIALS AND METHODS

Seeds of rice cultivar, Tainung No.67, were sown in plastic trays (size, 0.30 m×0.22 m ×0.06 m) containing vermiculite and watered daily with pure water. Seedlings were grown in an ambient environment with daily photoperiod of ca. 13 h, temperature of 25°C in the light, and 20°C in the dark. Pure water was made from a RO Pure ST reverse osmosis plus NANO Pure ultrapure water system (model D4741, Barnstead/Thermolyne Corp., USA) with electrical conductivity of 0.9 S m^{-1} and pH of 7.97. The 2-to 3-wk-old seedlings were

divided into groups of five. Enough plants were randomly selected from each group and their roots were subjected to a range of simulated acidic medium treatments (Table 1) for 1h. The pure water treatments were used as control. Simulated acidic media at pH's of 2.5 to 5.0 were made up by additions of sulphuric and nitric acids in a ratio of 1:1. 5:1 or 1:5 by volume to pure water.

Table 1. The composition and pH of simulated acid rain[z].

pH	Ratio	H_2SO_4(ml L^{-1})	HNO_3(ml L^{-1})
2.5	1 : 1	10.859	16.902
	5 : 1	18.098	5.634
	1 : 5	3.620	28.170
3.0	1 : 1	3.569	5.555
	5 : 1	5.965	1.825
	1 : 5	1.173	9.284
3.5	1 : 1	1.173	1.825
	5 : 1	1.927	0.603
	1 : 5	0.388	3.000
4.0	1 : 1	0.377	0.587
	5 : 1	0.627	0.198
	1 : 5	0.128	0.976
4.5	1 : 1	0.102	0.159
	5 : 1	0.161	0.050
	1 : 5	0.037	0.291
5.0	1 : 1	0.040	0.062
	5 : 1	0.066	0.020
	1 : 5	0.013	0.103

[z] The concentration of stock solutions for H_2SO_4 and HNO_3 is 0.1 M.

The treated plants were washed free of vermiculite with pure water and then separated into the aboveground plant portions and roots for investigation of their interaction to simulated acidic media. There were 5 replicates for each experiment and the experiment was repeated three times. Statistics was applied for comparisons of treatment effects. Samples of 0.5 g f.w. of rice leaves or roots were rinsed with pure water three times, air-dried, and ground in liquid nitrogen. The fine powder was homoginized with 100 ml of pure water for subsequent pH and electrical conductivity measurements.

Electrical comductivity was measured at about 25°C with a calibrated conductivity meter (model 32, YSI Inc., USA) after well stirring. Relative conductivity (RC) was calculated from: RC= (Ci/Cc) ×100%, where Ci and Cc are conductivities of simulated acid medium treatments and the control, respectively. The value of pH was measured by a pH meter (model 761, Knick Inc., Germany). For acidic buffering capacity determination, leaf and root homogenates were made from 1 g f.w. samples, ground in liquid nitrogen, and 30 ml pure water was added. Solutions were titrated to bring pH down an unit with 0.02 N HCl. Titration was achieved under stirring and pH changes were recorded.

RESULTS AND DISCUSSION

Acid rain (or acidic medium) is mainly pass through the cuticle into leaf rather than via stomata[7,14,29]. The cuticle therefore acts as the primary barrier to diffusion of non-gaseous substances into leaves and to leaching of solutes from the tissue[20,21,35,38]. Smalley *et al.*[30] pointed out that acids permeate leaf cuticle through charged pores, which function as a fixed buffer delaying, not inhibiting, acid movement. From the experimental results graphed in Fig. 1, the anionic form of acidic ions (HSO_3^-,NO_3^-) may release protons (H^+)

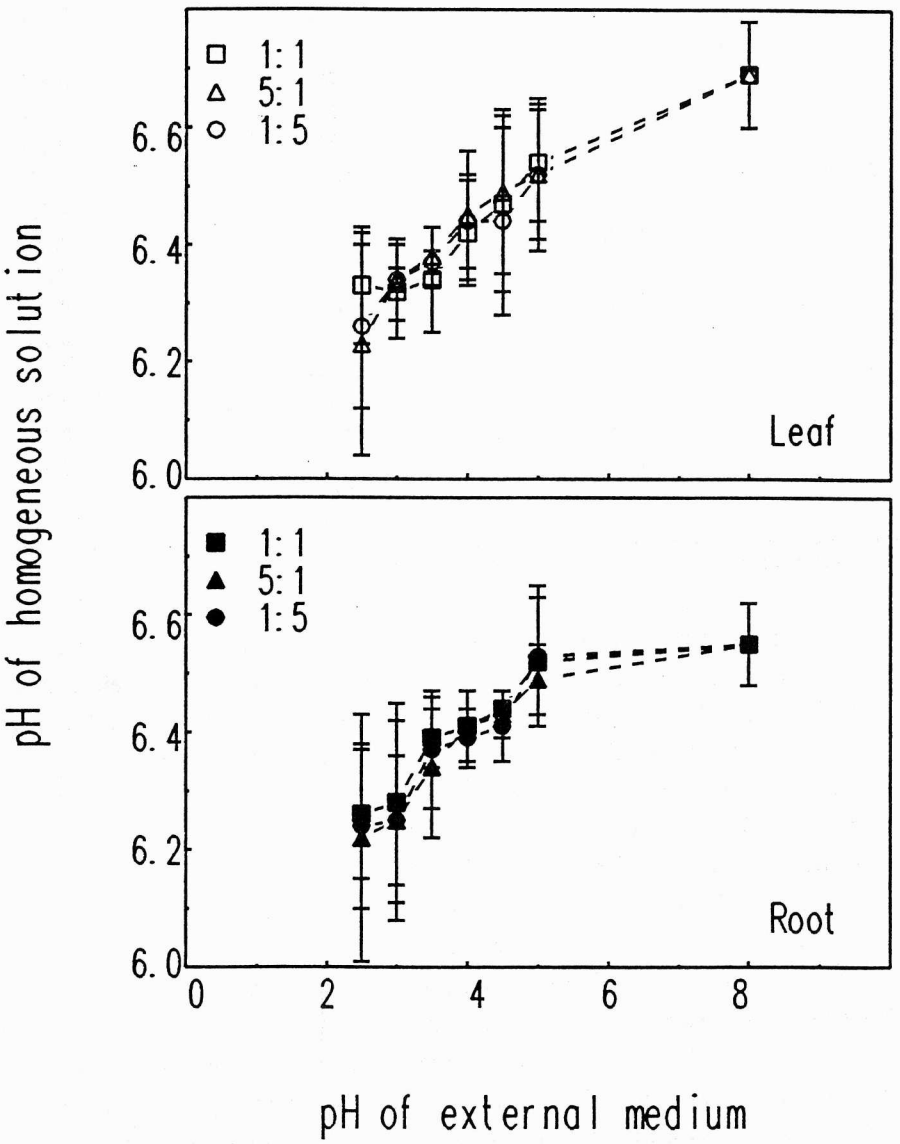

Fig. 1. Correlations between pH of homogeneous solution and external medium. The standard error is included.

when trapped by the cells to decrease cytoplasmic pH. Since the magnitude of flux gradients from the external medium into the cells depends on the balance between intracellular and extracellular pH[24], it was not abnormal to have lower cytoplasmic pH at the extremes of external pH (Fig. 1). The anionic species inside the cells will consume hydroxyl ions and decrease the pH of aqueous phases.

Raven[25] mentioned that phototrophs as a group can carry out their normal growth and maintenance activities over the external pH range 1-11. Smith and Raven [31] and Kuridjian and Guern[16] also showed that the cytoplasmic pH of the plants and bacteria was relatively insensitive to changes in external pH. As shown in Fig. 1 and Table 2, rice plant can regulate cytoplasmic pH to a certain extent, at least in the short term of soaking treatment, even when external pH is unsuitable for growth (down to 2.5). Cytoplasmic pH of rice leaf was decreased only two tenths of unit (6.69 to 6.45-6.42) when external pH was lowered down 4 units (7.97 to 4.0). Averagely, intracellular pH only changes by 0.1 units per 2 units change in the medium. Similarly, it fell about one tenth of unit (6.55 to 6.44-6.41) of homogenate per 2 units (7.79 to 4.5) decrease extracellularly for rice root. But root was more sensitive to external pH than leaf, pH 4.5 to pH 4.0.

Table 2a. Electrical condutivity, pH, and acidic buffering capacity of homogeneous solutions of leaves of rice, cultivar Tainung No.67, treated with simulated acidic media.

pH	H_2SO_4/HNO_3 ratio	Electrical comductivity (10^2 Sm^{-1})	pH	Acidic buffering capacity (ml g^{-1} f.w.)
8.0	CK	2.527±0.242[z] (100)[y]	6.69±0.09(100)	1.011±0.031(100)
5.0	1 : 1	2.647±0.122 (105)	6.54±0.10(98)	1.009±0.029(100)
	5 : 1	2.710±0.145 (107)	6.52±0.11(97)	1.011±0.015(100)
	1 : 5	2.762±0.201 (109)	6.52±0.13(97)	1.012±0.011(100)
4.5	1 : 1	3.027±0.309 (120)	6.47±0.15(97)	1.117±0.136(110)
	5 : 1	2.904±0.249 (115)	6.49±0.14(97)	1.069±0.148(106)
	1 : 5	2.822±0.194 (112)	6.44±0.16(96)	1.088±0.120(108)
4.0	1 : 1	3.021±00315 (120)	6.42±0.09(96)	1.122±0.153(111)
	5 : 1	3.063±0.309 (121)	6.45±0.11(96)	1.132±0.138(112)
	1 : 5	2.965±0.175 (117)	6.44±0.08(96)	1.119±0.163(111)
3.5	1 : 1	3.135±0.316 (124)	6.34±0.09(95)	1.154±0.199(114)
	5 : 1	3.095±0.258 (122)	6.38±0.05(95)	1.152±0.174(114)
	1 : 5	3.127±0.044 (124)	6.37±0.02(95)	1.134±0.163(112)
3.0	1 : 1	3.188±0.613 (126)	6.32±0.08(94)	1.138±0.075(113)
	5 : 1	3.233±0.139 (128)	6.34±0.02(95)	1.161±0.044(115)
	1 : 5	3.126±0.216 (124)	6.34±0.07(95)	1.159±0.094(115)
2.5	1 : 1	3.160±0.636 (125)	6.33±0.10(95)	1.150±0.124(114)
	5 : 1	2.981±0.423 (118)	6.23±0.19(93)	1.164±0.130(115)
	1 : 5	3.089±0.416 (122)	6.26±0.14(94)	1.153±0.085(114)

[z] mean±S.E.
[y] percent of the control

Table 2b. Electrical condutivity, pH, and acidic buffering capacity of homogeneous solutions of roots of rice, cultivar Tainung No.67, treated weth simulated acidic media.

pH	H_2SO_4/HNO_3 ratio	Electrical comductivity (10^2 Sm^{-1})	pH	Acidic buffering capacity (ml g^{-1} f.w.)
8.0	CK	1.030±0.023[z] (100)[y]	6.55±0.07(100)	0.478±0.047(100)
5.0	1 : 1	1.066±0.078 (103)	6.52±0.11(100)	0.518±0.015(108)
	5 : 1	1.063±0.083 (103)	6.49±0.06(99)	0.538±0.032(113)
	1 : 5	1.063±0.095 (103)	6.53±0.12(100)	0.546±0.076(114)
4.5	1 : 1	1.109±0.112 (108)	6.44±0.03(98)	0.555±0.032(116)
	5 : 1	1.074±0.130 (104)	6.43±0.04(98)	0.542±0.052(114)
	1 : 5	1.085±0.149 (105)	6.41±0.06(98)	0.543±0.036(114)
4.0	1 : 1	1.192±0.135 (116)	6.41±0.06(98)	0.605±0.044(127)
	5 : 1	1.165±0.036 (113)	6.41±0.06(98)	0.594±0.083(124)
	1 : 5	1.150±0.087 (112)	6.39±0.05(98)	0.591±0.006(124)
3.5	1 : 1	1.203±0.084 (117)	6.39±0.05(98)	0.640±0.100(134)
	5 : 1	1.158±0.153 (112)	6.34±0.12(97)	0.643±0.074(135)
	1 : 5	1.200±0.091 (117)	6.37±0.10(97)	0.626±0.019(131)
3.0	1 : 1	1.350±0.206 (131)	6.28±0.17(96)	0.722±0.032(151)
	5 : 1	1.360±0.078 (132)	6.25±0.11(95)	0.735±0.028(154)
	1 : 5	1.334±0.161 (130)	3.25±0.17(95)	0.718±0.032(150)
2.5	1 : 1	1.318±0.210 (128)	6.26±0.11(96)	0.757±0.013(158)
	5 : 1	1.352±0.311 (131)	6.22±0.21(95)	0.779±0.028(163)
	1 : 5	1.342±0.282 (130)	6.24±0.14(95)	0.765±0.050(160)

[z] mean±S.E.
[y] percent of the control

At present, the only detailed study on a phototroph, *Chara corallina,* suggests a constancy in the charge state of the sum of organic electrolytes[26]. That is, maintenance of cytoplasmic pH is regulated by the ionization state of the $[CO_2]$ / $[HCO_3^-]$ system. But even with a constant electrochemical potential difference for H^+ at the plasmalemma, behaving like the artificial bilayer *in vivo,* at all pH values, the unregulatable H^+ leak into the cell is higher at extremes of extemal pH[13]. As demonstrated in *Chara corallina,* the H^+-pumping state of actively transport and the passive H^+ flux state are co-exist in this organism, H^+ pumping is activated in the acid zones and passive H^+ fluxes in the alkaline zones[1,2,3,4,5]. If that is the case in rice seedling, H may flux from the external medium of lower pH into rice cells of higher pH through the regulation of this dual function. However, adjustment of intracellular pH of cells to near neutrality is also required to maintain an equilibrium state for metabolism to be continued. How rice plant to maintain its cells in near neutrality is the unknown part yet to be clarified. On the other hand, H co-transport systems may be very sensitive to cytoplasmic pH so that small but significant changes in cytoplasmic pH rapidly stabilize rates of co-transport at low or high external pH values[27]. This might be an explanation for the regulated decrease in cytoplasmic pH of 0.1 units per 2 units decrease in external pH, which is a common feature occurring in the natural environment.

As shown in Fig. 2, changes of electrical conductivity of homogenates were in accor-

dance with that of pH. As data listed in Table 2, a 22-24% of significant increase of electrical conductivity was found in leaf homogenate when the pH of acidic media lowered down to 3.5. At pH 4.0, electrical conductivity of root homogenate went up 12-16%. Again, the sensitivity of root was higher than leaf (pH 4.0 relative to pH 3.5), but the magnitude was in opposite. From the titration curves (Fig. 3), acidic buffering capacity was also strongly pH-dependent. The cytoplasmic buffering capacity of rice leaf and root was deducible, high

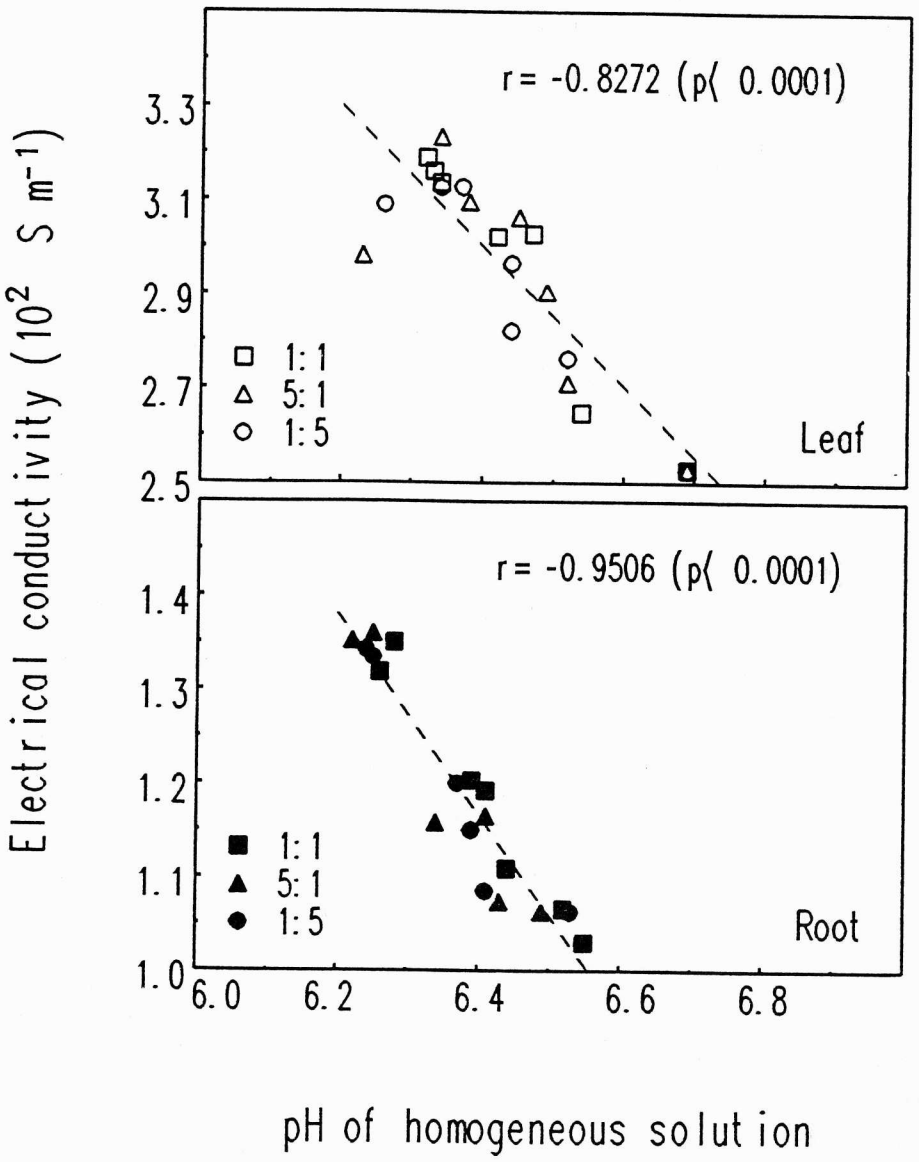

Fig. 2. Changes of eletrical conductivity of homogeneous solution in response to pH value. Correlation coefficient is included.

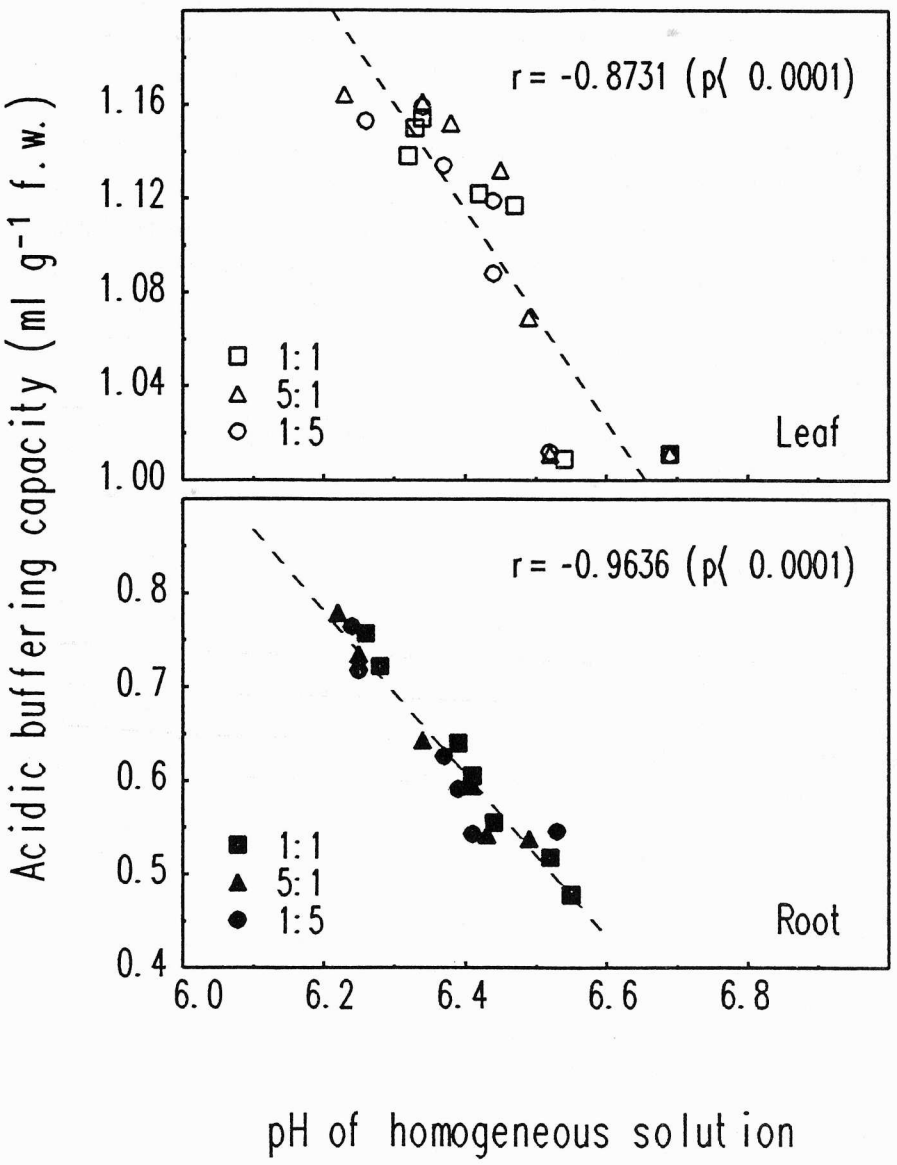

Fig. 3. Changes of acidic buffering capacity of homogeneous solution in response to pH value. Correlation coefficient is includes.

at low cytoplasmic pH. Together with the performance of electrical conductivity, it implies that the rate of proton production or hydroxyl ion consumption and of the buffering capacity of aqueous phases of rice cells can be promoted by lowering external pH level. But the kinds of electrolytes and solutes making the increase of electrical conductivity and acidic buffering were not analyzed in this study. Experimental results of Scholz and Knabe[28] and Thomas *et al.* [34] also indicated that, in addition to metabolic reactions for stabilizing

cytoplasmic pH, the rate of the decrease of pH in the cells is a function of the rate of proton generation (or dydroxyl ion depletion) and buffering capacity. Pfanz and Heber[24] showed that the cytoplasm was much more buffered than the vacuole when compared on a unit volume basis. With its acidic nature, the vacuole possesses little trapping potential than cytoplasm in spite of its large size.

It is concluded from this study that the concentration of simulated acid rain (medium) must be increased to an extent causing an acidification of cytoplsm to decrease intracellular pH. The capacity of acidic buffering and the mechanism(s) involved are still unclear and require in depth invertigation. Interestingly, at the same level of pH no statistically significant difference was found within acidic medium treatments of different compositions. How different components interact to exert on same impact is another question needs to be solved.

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酸雨對水稻生長與產量之影響

(一) 人工模擬酸雨組成份及酸鹼值之差別效應[1]

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摘要：酸雨或稱酸性濕沉降（acid precipitation），隨著其組成份、濃度、及淋洗期長短，可能對農作物造成不等程度的危害。本研究乃以不同組成份之不等酸鹼值人工模擬酸雨（simulated acid rain）浸潤水稻幼株根部1小時，藉以探討不同酸雨組成對若干生理性狀之差別反應。試驗以播種2～3週的稻苗（臺農67號）爲試材，1：1、5：1、1：5等三種不同硫酸（H_2SO_4）與硝酸（HNO_3）比例之試液爲人工模擬酸雨，並調製成2.5、3.0、3.5、4.0、4.5、及5.0等六種酸鹼值等級，而以純水處理爲對照組，比較葉片與根系均質液之酸鹼值、電導度值、及酸緩衝量之差異。根據試驗結果，當人工模擬酸雨酸鹼值低至4.0時，葉片均質液之酸鹼值即呈現明顯下降(惟降幅甚小，僅由 pH 6.69至 pH 6.45～6.42）；當人工模擬酸雨續降至 pH 3.5時，葉片均質液之電導度值顯著升高22～24%；再降至pH 3.0時，酸緩衝量將顯著上升13～15%。根系均質液亦在人工模擬酸雨酸鹼值達4.5時，其 pH 呈明顯差異（惟仍僅由6.55降爲6.44～6.41）；而當酸雨之酸鹼值續降至4.0時，根系均質液電導度值即上升12～16%；再降至 pH 3.5時，酸緩衝量則顯著增加31～35%。由此可見，稻苗根系對人工模擬酸雨酸鹼值的敏感程度高於葉片，惟其反應幅度不一。試驗結果亦顯示，在同一酸鹼值本試驗採用之三種酸雨組成份處理之間並未對所調查稻苗生理性狀產生顯著差異。

關鍵詞：水稻幼株、人工模擬酸雨、酸鹼值、電導度值、酸緩衝量、均質液。

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